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The Use of Roots to Test N, P and K Deficiencies in *Eucalyptus* Nutrition

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SYNOPSIS

Bioassays have been developed which can provide diagnostics of the nutritional status of trees. They measure the flux of N, P or Rb for K into roots from standard solutions where the rate of influx is inversely related to nutrient supply. The bioassays have been applied in the study of nutrient deficiencies in *Eucalyptus grandis* in both pot and field trials, and showed greater sensitivity than foliar analysis in detecting nutrient deficiencies and interactions between elements which may influence growth and wood production. The way in which these bioassays could be used as a management tool is discussed.

Keywords: *Eucalyptus grandis*, root bioassays, nutrition, foliar analysis.

BACKGROUND

Development of bioassays

In commercial forestry, as in agriculture, there is a financial need to optimise yield in relation to expenditure on fertiliser. In most agricultural soils, fertiliser requirement can be assessed by chemical analysis of soil to detect deficiencies of elements. The chemical techniques used in fertile agricultural soils do not work well for poorer soils used for plantation forestry because the availability of nutrients in these soils is often more dependent on cycling rates rather than nutrient pool size.

As many British soils are phosphate limiting, a root bioassay to determine phosphate status of trees has been developed by Harrison and Helliwell (1979) which is easy to perform and related to both estimates of phosphate availability in soil and demand by the plants. This bioassay is based on the "hunger" response of roots as measured by the rate of influx of phosphate into roots (intact or excised) from a standard phosphate solution. As this influx rate is below detection by chemical methods, the radiotracer 32P is used. The general response is curvilinear, often approximating to a negative exponential function. Uptake of phosphate from solution is high in roots grown in P deficient conditions, and low in roots from high levels of supply (Harrison and Helliwell, 1979). The bioassay has successfully demonstrated the phosphate status of tree seedlings growing in pots under defined nutrient regimes (Harrison and Helliwell, 1979) and in the field under different fertiliser treatments (Dighton and Harrison, 1983). Subsequently, bioassays have been developed, based on the same principle, for potassium, using 86Rb tracer (Jones *et al.*, 1987) and for nitrogen using the stable isotope 15N (Jones *et al.*, in press). The generalised response curve of bioassay nutrient uptake by roots with nutrient supply is shown in *Figure 1*.

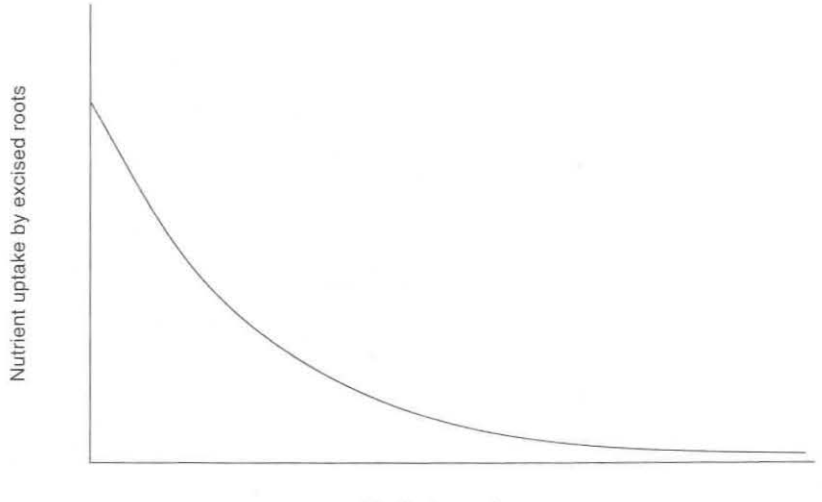

FIGURE 1. Generalised response curve of roots in the bioassays: influx of nutrient element into root in relation to nutrient supply.

Advantages of the root bioassay

From the work carried out, primarily using the phosphate bioassay, it appears that the method is able to detect the roots' ability to integrate soil nutrient supply and plant demand for that element. Nutrient demand by the plant is determined using the organ of uptake (the root) which is probably more related to the overall nutrient status of the plant than an element in a nutrient sink (the leaf). There is, however, an overall relationship between the bioassay assessment of nutrient status and foliar concentration of the element (Dighton and Harrison, 1983). However, this is demonstrated only when plantations of very poor and very good nutritional status are included in the same regression; otherwise the fit is poor. In trials of the phosphate bioassay in temperate upland plantations of Sitka spruce (*Picea sitchensis* (Bong). Carr) the bioassay has appeared to be more sensitive than foliar chemical analysis. Roots have been shown to respond to fertiliser additions within one month, a response not detected in foliage in that time (Dighton and Harrison, 1983). In an analysis of nutrient status of *Picea sitchensis* plantations of increasing age, a mid-rotation phosphate deficiency was demonstrated with the bioassay, although it was not evident from foliar analysis (Dighton and Harrison, 1990). An additional advantage of the technique is the simplicity of the procedures used. The mechanics of running root bioassays are simple and rapid, although there is need for sophisticated equipment such as a liquid scintillation counter (32P and 86Rb) and mass spectrometer (15N). Roots are able to continue to respond in the bioassays after storage for 72 h, but there is a need for physiological integrity to be maintained.

The phosphate bioassay has been shown to have applicability in a wide range of plant species; tree genera *Betula* and *Acer* (Harrison and Helliwell 1979), *Picea* and *Pinus* (Dighton and Harrison 1983; 1990), the grasses *Agrostis* and *Festuca* (Harrison *et al.* 1984; 1991) and the cereals *Triticum* and *Hordeum* (Harrison and Dighton 1990). The nitrogen bioassay has also been applied successfully to a range of plant genera; *Agrostis, Betula, Pinus, Picea* (Jones *et al.* in press, unpublished data). The phosphate bioassay has also been applied to citrus plantations in South Korea and Bazil (Dighton unpublished data; Harrison unpublished data). The bioassay indicated there would be no response to applied P fertiliser due to the loss of applied P by chemical fixation in the soils as the sites were on soils of high P fixing capacity.

The advantages of having bioassays for each of the three major elements (N, P and K) allow us not only to determine deficiencies of single elements but to explore the interactions between them. The consequence of nutritional imbalance as a result of addition of one or two elements can be assessed. This information can be used to determine future forest management options.

Applicability of the bioassays to *Eucalyptus grandis* plantations

All three bioassays were applied to a fertiliser trial of *Eucalyptus grandis* Hill ex Maiden in coastal Natal, South Africa. Trees were planted in September 1989 and given two rates of N, P and K fertiliser in a factorial design with two replicate blocks per treatment. Trees were given half of the fertiliser at planting and half at either 3 or 6 months (time of application). Each major nutrient element was added at one of two levels, N at 70 or 140 kg/ha as limestone ammonium nitrate; P at 40 or 80 kg/ha as superphosphate and K at 20 or 40 kg/ha as potassium sulphate. All combinations of the nutrients at each level were included together with controls of no addition. Superimposed on this arrangement was a trace element addition at one of two levels. For the sake of simplicity, the effect of trace element addition will not be discussed in this paper.

Tree heights given here were measured at 6 months of age, and the bioassays were carried out 12 days (P and K) and 18 days (N) after the 6 month application of fertiliser. Leaf samples for foliar analysis were taken 5 days after the 6 month fertiliser addition. *Table 1* shows the effects of fertiliser treatment on growth and nutritional status of the crop. Tree height and bioassay nutrient uptake data were analysed by factorial analysis of variance for significant differences in relation to fertiliser treatment. Tree height was most influenced by level of nitrogen addition and this was reflected in both an increase in foliar N reduction in bioassay N uptake at the higher levels of addition. Nitrogen has been shown to be limiting for *Eucalyptus* by Donald and Schutz (1977), Thivolle-Cazat (1986) and Grove (1988).

In the bioassays, higher rates of addition of both P and K also reduced the uptake of these elements, although this was only statistically significant for P. There was, however, no difference in the P and K concentrations in the leaves with addition of fertiliser. Indeed, the stunted control trees had higher foliar levels of P and K than trees receiving either level of fertiliser addition. Foliar concentrations of all elements in all treatments including the control would be considered satisfactory according to current recommended values (Schönau and Herbert, 1982). Interestingly, the addition of high fertiliser rates of both P and K resulted in a suppression of bioassay N uptake compared to the low, suggesting enhanced N uptake by the trees. This would indicate some synergistic coupling of uptake in order to maintain nutrient equilibrium (Ingestad, 1979; Ingestad and Lund, 1979). This may, in due course, be reflected by better growth in these treatments. It may be an early sign of the overall improved nutrition which is indicated by the reduced N uptake.

Table 2 shows the effect of application of fertiliser at different times after planting. Tree heights show that an early second application of fertiliser was beneficial. Foliar N showed a slight and significant increase following the six month fertiliser addition, although there was no increase in foliar P content. Both the bioassay N and P uptake were reduced as a result of the six month addition of fertiliser. The experimental plantation in Natal had only two rates of fertiliser addition, so it was not possible to obtain a response curve or to study adequately the interaction between the elements. Previously we had carried out the bioassays on *E. grandis* seedlings in a 3^3 fac-

TABLE 1. E. grandis *response to differential fertiliser addition in a field experiment in coastal Natal. Values given are means*

	Nitrogen			Phosphorus			Potassium		
Fertiliser added (kg/ha)	**0**	**70**	**240**	**0**	**40**	**80**	**0**	**20**	**40**
Tree height (m)[a]	1,83	2,49 ***	2,73	1,83	2,58	2,64	1,83	2,63	2,58
Element in foliage[b] (%)	2,80	2,96 *	3,14	0,27	0,26	0,26	1,30	1,15	1,13
N uptake (ng N/mg root/2 h)[n]	39,6	31,9 ***	22,6	39,6	28,3 *	26,2	39,6	28,8 **	25,8
P uptake (pg P/mg root/15 min)[n]				602	1 119 *	403			
K uptake (pg Rb/mg root/15 min)							165	229	154

Significant differences between values joined by a line within rows are denoted for [a], ANOVA and [b], Wilcoxon Rank Test by *, P <0,05; **, P <0,01 and ***, P <0,001.

torial N, P and K pot experiment at the Shell Research Centre in Sittingbourne, Kent, UK. From this, it was possible for us to study in more detail the interactions between nutrients and *E. grandis* seedling growth.

TABLE 2. Effect of time of fertiliser addition on E. grandis *growth, foliar nutrient content and index of nutrient deficiency determined within one month of second fertiliser addition. Data are means over all levels of addition of fertiliser.*

	Fertiliser regime		
	At planting and 3 months	**At planting and 6 months**	
Tree height	2,7	2,5	**[a]
Foliar N (%)	2,96	3,14	*[b]
N uptake (ng N/mg root h $^{-1}$)	29,8	24,7	***[a]
Foliar P (%)	0,6	0,27	NS[b]
P uptake (pg P/mg root 15 min $^{-1}$	1 088	434	*[a]

Differences between means estimated from [a]ANOVA, [b]Wilcoxon Rank Tests (see Table 1).

As in the field, N was found to be the major limiting factor for *E. grandis* seedling growth *(Table 3)*. Addition of increased amounts of N increased foliar concentrations of that element and also significantly increased foliar concentration of P and K (which peaked at the intermediate level of N addition). There was a difference in behaviour between the seedling trees and six month-old trees in Natal. In the field, addition of P and K appeared to improve N nutrition. In seedling trees, addition of N caused a significant increase in demand for K and, to some extent, P. This could be ascribed to the high demand for N by seedlings, the growth response to which leads to deficiencies of other elements (Ingestad, 1979; Ingestad and Lund, 1979). With additional N supply, however, foliar K concentration increased along with foliar P *(Table 3)*. The link between induced root deficiency for K and increased foliar K with added N may be due to preferential movement of highly mobile K to sinks suggesting that foliar analysis may not be the best estimate of overall nutritional status. Addition of increased K reduced both K and P demand of the seedlings in the same way that increased P and K supply reduced N deficiency in field-grown trees.

TABLE 3. Effect of level of nutrient supply to E. grandis *seedlings in artificial media in a 3^3 factorial N, P. K interaction pot experiment after 4 weeks growth under the imposed nutrient levels. Means of parameters are shown only where they were significantly different between different levels of nutrients*

	N			P			K		
Nutrient supply (mg/1)	**10**	**100**	**250**	**10**	**20**	**50**	**25**	**100**	**250**
Height (mm)	96	127	131						
Stem: leaf	0,18	0,20	0,26	0,22	0,19	0,21			
N uptake (ng N.mg root 2 h $^{-1}$)	79	64	40						
P uptake (pg P/mg root 15 min $^{-1}$)				91	66	60	87	68	62
K uptake (pg Rb/mg root 15 min $^{-1}$)	46	65	102				129	51	33
Foliar N (%)	2,4	4,3	5,3						
Foliar P (%)	0,36	0,50	0,52	0,43	0,47	0,48			
Foliar K (%)	1,12	1,53	1,49				0,93	1,37	1,84

The pot study shows that there is a significant relationship between nutrition and stem:leaf ratio *Table 4)*. In general, this ratio increased significantly with increased N supply. Increased supplies of P only enhanced stem:-leaf ratio at high levels of N (N × P interaction is significant; F = 13,08, P <0,001). At low and intermediate levels of N, addition of P at intermediate and high levels reduced the stem:leaf ratio compared with the low levels of P. Intermediate levels of P had the strongest effect in reducing the stem:leaf ratio. Increased levels of K only increased stem:leaf ratio at intermediate or high levels of N (N × K interaction significant; F = 4,59, P <0,05).

TABLE 4. Interactions between N and P and N and K in the stem:leaf ratio of E. grandis *seedlings grown in artificial media for 4 weeks under different nutrient concentrations. Values of stem:leaf ratio are means of 4 replicates*

		N supply (mg/1)		
		10	**100**	**250**
P supply (mg/1)	10	0,22	0,24	0,22
	20	0,14	0,16	0,27
	50	0,16	0,19	0,29
K supply (mg/1)	25	0,18	0,20	0,25
	100	0,18	0,16	0,25
	200	0,17	0,23	0,28

This may be of importance in determining the nutrient balance for optimising wood production in forest stands.

IMPLEMENTATION OF BIOASSAYS AS MANAGEMENT TOOLS

At present, the bioassays are being used mainly as research tools in studies relating plant growth and nutrition to soil nutrient characteristics. However, there is considerable potential in these techniques for improving on current methods in the detection of nutrient deficiencies, the prediction of fertiliser requirements and thus the economic management of plantation forests.

In order to forecast the effects of fertiliser addition on forest growth, a number of factors have to be taken into account. *Figure 2* outlines some of the major factors influencing availability of nutrients in soil and nutrient demand by trees. The use of bioassays in the way we are applying them to *Eucalyptus* can provide considerable information on the response of trees to fertiliser and go some way to indicating optimal fertiliser requirements. However, in order to develop precise rules predicting tree growth response to fertiliser addition, other factors must be quantified and used to create an appropriate data base. These include the results of a number of trial experiments where the difference between bioassay nutrient uptake values between fertilised and non-fertilised stands have been translated into observed growth/yield increases. An instantaneous measure of difference between bioassay nutrient uptake between a small patch of fertilised forest and the unfertilised forest could then be used as input to the model to predict a growth/yield increase in response to the fertiliser *(Figure 3)*.

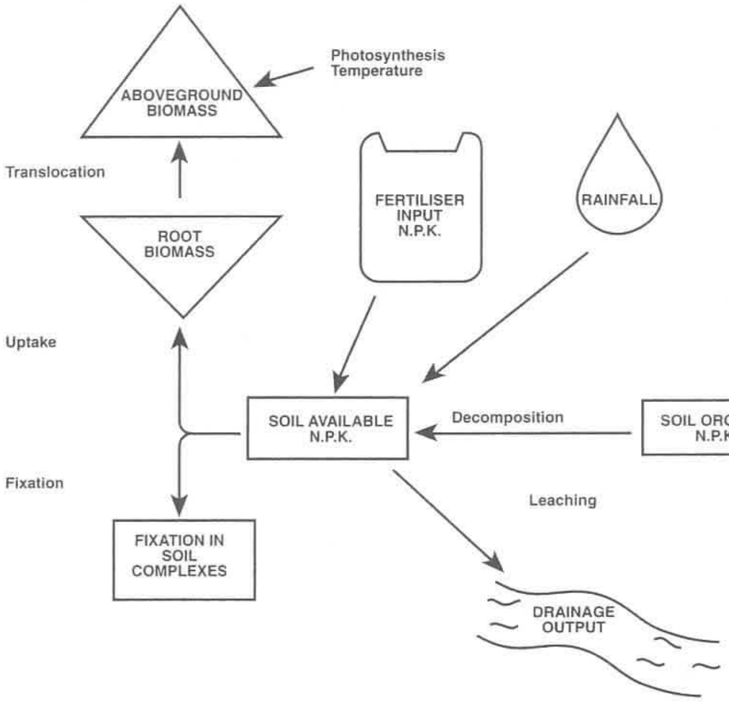

FIGURE 2. Schematic diagram of nutrient fluxes in forest trees. The interpretation of forecasts of potential growth increase after fertiliser addition will be influenced by these factors.

The prediction could be modified and refined depending on the additional information available for the site in question. Key factors are the soil characteristics which might alter the availability of fertiliser nutrient through leaching or fixation. The demand (sink strength) of the crop may change with stand age in relation to changes in the ratio of organic:inorganic nutrients in soil as a result of immobilisation processes (Dighton and Harrison, 1990). By bringing together a data base of information from a series of plantations, we can develop rules relating fertiliser requirement to site characteristics, site management and tree age/development.

As we are only beginning to look at *Eucalyptus* we are not yet in a position to give precise recommendations for fertiliser use, but we believe that the bioassays will fulfill this function. They are already providing to be more sensitive than foliar analysis. Our experience in temperate regions suggests that they can detect plant re-

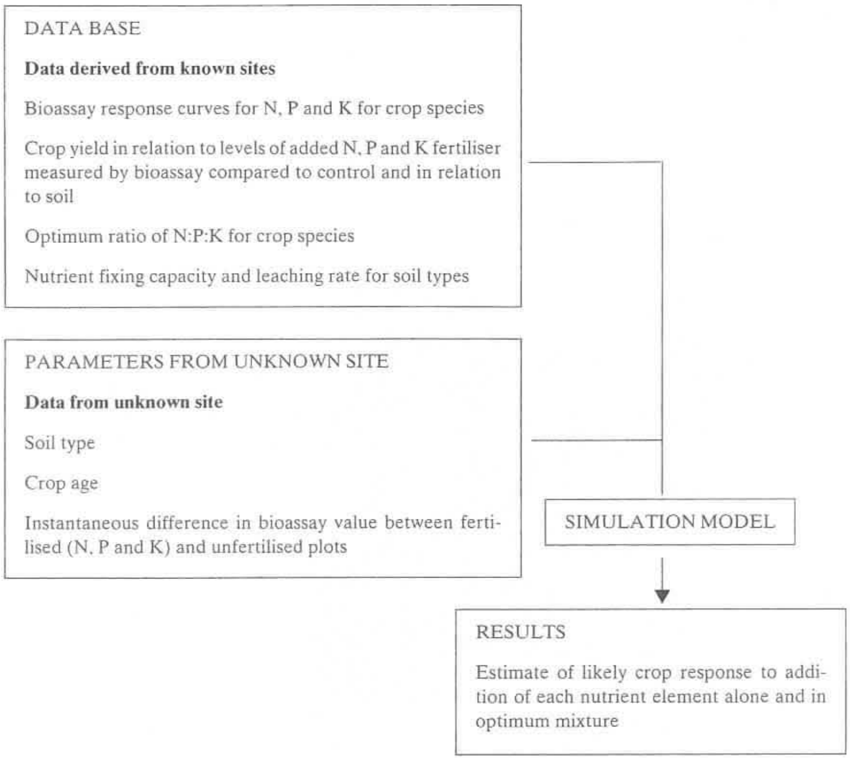
DATA BASE
Data derived from known sites
Bioassay response curves for N, P and K for crop species
Crop yield in relation to levels of added N, P and K fertiliser measured by bioassay compared to control and in relation to soil
Optimum ratio of N:P:K for crop species
Nutrient fixing capacity and leaching rate for soil types
PARAMETERS FROM UNKNOWN SITE
Data from unknown site
Soil type
Crop age
Instantaneous difference in bioassay value between fertilised (N, P and K) and unfertilised plots
SIMULATION MODEL
RESULTS
Estimate of likely crop response to addition of each nutrient element alone and in optimum mixture

FIGURE 3. Flow diagram of possible structure and use of bioassay procedure for estimating crop response to fertiliser addition.

sponse to changes in nutrient availability much more rapidly than foliar analysis.

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